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1 **Determinants of plasma alkylresorcinol concentration in Danish postmenopausal**
2 **women^{1,2,3}**

3 RIKARD LANDBERG^{a4}, AFAF KAMAL-ELDIN^a, PER ÅMAN^a, JANE CHRISTENSEN^b, KIM
4 OVERVAD^c, ANNE TJØNNELAND^b & ANJA OLSEN^b

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9 ³ Abbreviation used: AR: alkylresorcinol(s), DCH (Diet, Cancer and Health study) GC-MS-SIM: gas
10 chromatography mass spectrometry single ion monitoring, FFQ: food frequency questionnaire

11 ^aDepartment of Food Science, Swedish University of Agriculture Science (SLU), Box 7051, SE-75007
12 Uppsala, Sweden

13 ^b Institute of Cancer Epidemiology, The Danish Cancer Society, Copenhagen, Denmark

14 ^c Department of Cardiology and Department of Clinical Epidemiology, Aalborg Hospital, Aarhus
15 University Hospital, Sdr Skovvej 15, Box 365, 9000 , Aalborg, Denmark

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17
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19 ⁴ To whom correspondence should be addressed.

20 Phone +46 (0) 18 67 20 46

21 Fax +46 (0) 18 67 29 95

22 E-mail: rikard.landberg@lmv.slu.se

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24

25 **ABSTRACT**

26 **Background:** Alkylresorcinols (AR), a group of phenolic lipids present in the outer parts of
27 wheat and rye grain kernels, have been suggested as biomarkers of whole-grain wheat and rye
28 intake. In this study we investigated potential determinants of plasma AR concentration in a
29 free-living population.

30 **Subjects/Methods:** Non-fasting samples from postmenopausal women enrolled in the Danish
31 Diet, Cancer and Health study (n=360) were selected. Diet was assessed using a semi-
32 quantitative FFQ and the association between food items likely to contain AR as well as
33 relevant non-dietary factors were studied by ANCOVA-models.

34 **Results:** The median AR concentration was 78 nmol/l (interquartile range (IQR) = 106.9
35 nmol/l). Intake of rye bread, identified as the main determinant, was associated with 87%
36 higher plasma total AR concentration per 100 g bread (95% CI= 46-139%). About 8-12 % of
37 the total variation (depending on AR homologue) in plasma AR concentration was explained
38 by the selected dietary variables. At a nutrient level, total dietary fiber and cereal fiber were
39 significantly associated with plasma total AR concentration (P=0.05), but only ≈2 % of the
40 total plasma AR concentration was explained by the dietary fiber or cereal fiber intake.

41 **Conclusion:** In the studied population, AR plasma concentration was mainly affected by rye
42 bread intake among investigated determinants.

43

44 **INTRODUCTION**

45 Epidemiological studies have consistently shown a reduction in risk of coronary heart disease
46 and Type 2 diabetes (Mellen et al 2008, Priebe et al 2008), and some studies have shown a
47 reduction in risk for certain cancers (Jacobs et al 1998). The mechanisms for the protective
48 effect(s) are poorly understood, but components within the dietary fibre complex are believed
49 to act together (Slavin 2003). A major obstacle in nutritional epidemiology, is the relative
50 large errors in the estimation of dietary and nutrient intake, which are often derived from
51 semi-quantitative food frequency questionnaires (FFQs) (Kaaks et al 1997).

52 Whole grain intake estimation is particularly likely to be subjected to measurement errors, due
53 to lack of uniform whole grain definition across studies (Koh-Banerjee et al 2004) and the fact
54 that consumers might have difficulties in discerning whole grain from non-whole grain foods
55 (Adams and Engstrom 2000). Moreover, FFQs usually contain few whole-grain food items
56 listed, and whole grain content in whole grain foods may vary considerably in different
57 products and hence, misclassification is likely (Fung et al 2002). A selective biomarker of
58 whole grain intake would have the potential to provide a more valid ranking tool of intake
59 than traditional dietary assessment methods since it is most likely independent of errors
60 associated with these methods (Kaaks et al 1997). Before use, the biomarker needs to be
61 evaluated and validated in both controlled studies and in randomly selected free living
62 subjects in order to get insights of its potential limitations (Hunter 1998, Landberg et al
63 2008b, van Dam and Hu 2008). In controlled studies, the pharmacokinetics (revealing the
64 time course of reflection) and response to changes in intake can be studied in detail, whereas
65 population-based samples can be used to estimate variation, determine long term
66 reproducibility, and to explore determinants of the biomarker concentration (Johnsen et al
67 2004, Kaaks et al 1997, Landberg et al 2009a, Montonen et al 2010, White 1997)

68 Alkylresorcinols (AR), a group of phenolic lipids, have recently been suggested as
69 biomarkers of whole grain wheat and rye intake, because they are exclusively present in the
70 outer parts of these cereals (Landberg et al 2008a, Landberg et al 2008c, Linko-Parvinen et al
71 2007, Ross et al 2004). Results from whole grain intervention studies have shown that AR
72 concentration increases with increased whole-grain intake in a dose-dependent manner and
73 that the concentration remains stable over weeks in people with frequent whole grain intake
74 (Landberg et al 2006, Landberg et al 2008a, Landberg et al 2008b, Landberg et al 2008d,
75 Linko-Parvinen et al 2007, Linko et al 2005). One recent study showed a modest correlation
76 ($r=0.4$) between cereal fibre intake assessed by 3 day-weighted food records and plasma AR
77 concentration in selected Finnish free living subjects (Aubertin-Leheudre et al 2008).
78 Moreover, a recent small study ($n=33$) compared whole grain intake (g/d) assessed by a FFQ
79 designed specifically for whole grain products and plasma total AR concentration and found a
80 good correlation ($r=0.57$) (Ross et al 2009). The reproducibility over a period of 4 months was
81 shown to be modest-good ($ICC=0.55$) for women in a subsample of the EPIC-Potsdam cohort
82 suggesting that plasma AR concentration is rather stable over time within an individual
83 (Montonen et al 2010). However, no study has explored possible determinants of plasma AR
84 concentration in a broad group of free living subjects. Such a study is an important step in the
85 evaluation of the selectivity of a biomarker. The aim of the present study was to estimate the
86 variation in plasma AR concentration and to determine dietary and non-dietary factors
87 affecting the plasma AR concentration in free living Danish postmenopausal women.

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93 **MATERIALS AND METHODS**

94 *Study population, data- and sample collection*

95 Between December 1993 and May 1997, 79 729 women who lived in the greater Copenhagen
96 and Aarhus area were invited to participate in the prospective cohort study “Diet, Cancer and
97 Health,” (DCH). A total number of 29 875 women that fulfilled the criteria were enrolled.
98 (Tjønneland et al 2007). At baseline, participants attended one of two established centers,
99 where 30 mL of blood (collected in citrated and plain Venojects) were drawn from each
100 participant. For practical reasons, subjects were not instructed to provide fasting samples time
101 since last meal was recorded at blood draw. Samples were centrifuged (2000 G for 10
102 minutes) and plasma was divided into 1 mL tubes which were stored in liquid nitrogen vapour
103 until analysis (maximum temperature -150°C).

104

105 Subjects filled in a 192-item FFQ and a lifestyle questionnaire, which they received by mail
106 before visiting the clinic to donate blood. The development and validation of the FFQ has
107 been described elsewhere (Haraldsdóttir et al 1994, Overvad et al 1991, Tjønneland et al
108 1991, Tjønneland et al 1992). The participants were instructed to report their intake of foods
109 and beverages during the past 12 months, within 12 possible categories ranging from never to
110 ≥ 8 times /d. Daily intakes of specific foods and nutrients were calculated for each participant
111 by the software program Food Calc (<http://www.ibt.ku.dk/jesper/foodcalc/>) using specially
112 developed standardized recipes and portion sizes (Johnsen et al 2004). Anthropometrical
113 measurements were carried out by professionals and the BMI was calculated as weight (kg)
114 divided by height squared (m^2).

115

116

117 A total number of 360 postmenopausal women (177 cases and 184 subcohort members) from
118 a nested case-cohort study on AR in relation to endometrial cancer (Olsen et al 2009) were
119 included in the present study. Because there were no statistically significant differences
120 between cases and the sub-cohort with regard to plasma AR concentrations (Olsen et al 2009),
121 both groups were pooled and included in the present cross-sectional analysis. Base-line blood
122 samples from all 360 women were analyzed for plasma AR concentration. The DCH study
123 and the present sub-study were approved by the regional ethical committees on human studies
124 in Copenhagen and Aarhus and by the Danish Data Protection Agency. All participants
125 provided written informed consent.

126

127 *Identification of potential plasma AR determinants*

128 Alkylresorcinols are only present in significant amounts in whole grain/bran of wheat and rye
129 and in Denmark a large proportion of the whole-grain intake comes from very specific food
130 items, particularly rye bread (Mejborn et al 2008). We therefore selected to investigate
131 potential determinants of plasma AR concentration among foods known to contain AR and
132 foods reflected by FFQ questions that have been used before to reflect whole grain intake in
133 this cohort (Egeberg et al 2009). The foods included rye bread, “other-bread” (breads that
134 contained whole grain wheat, were not purely refined and did not contain rye), crisp-bread
135 and refined bread. Danish rye bread, “other-bread” and rye crisp bread (which contributes to
136 about 50% of the total crisp bread intake) typically contains 32, 7.5 and 83% whole grain on
137 fresh weight basis, respectively (Mejborn et al 2008). However, the variation in whole grain
138 content and, hence, also AR content within these food items is high. Refined bread was
139 included because it was recently shown to contain very small amounts of AR (Ross and
140 Kochhar 2009). We also investigated beer, which contains traces (<30 µg/g on dry matter
141 basis) of AR (unpublished results) and total fat derived from animal sources as predictors of

142 AR concentration. We recently showed that AR homologues are accumulated in adipose
143 tissue in pigs and humans (Jansson et al 2010). Due to a short apparent elimination half-life
144 (about 5 h) and potential accumulation of AR in adipose tissue in case dietary sources are
145 frequently consumed, time since last meal and BMI were tested as predictors as well. Serum
146 cholesterol was included because AR are transported in lipoproteins (Linko-Parvinen et al
147 2007).

148

149 *Analytical methods*

150 Plasma samples (300 μ L) were analyzed for AR homologues C17:0-C25:0 according to a
151 recently published GC-MS-SIM method, using molecular ions for quantification (Landberg et
152 al 2009b). Samples were analyzed randomly in singlicates in 17 batches by 3 different people.
153 In each batch, five replicates of a control sample were included randomly in the sequence.
154 The within- and between day batch variation determined as the coefficient of variation (CV)
155 was <7% and <10%, respectively.

156

157 *Statistical analysis*

158 For the statistical evaluation, ANCOVA models with AR as the dependent variable were used
159 and results where $P < 0.05$ were considered significant. Since plasma AR concentration was
160 skewed, data were transformed by natural logarithm before analysis and estimates were then
161 back transformed to illustrate percentage change in AR concentration. Statistical analysis was
162 combined for cases and controls ($n=360$) since no significant differences were observed
163 ($P=0.76$ (total AR), $P=0.54$ (C17:0), $P=0.85$ (C19:0), $P=0.53$ (C21:0), $P=0.67$ (C23:0) and
164 $P=0.96$ (C25:0)). All quantitative variables were entered linearly in the model (Greenland
165 1995b). The women were grouped in quartiles according to their plasma total AR
166 concentrations and the distribution of selected dietary and non-dietary variables was

167 calculated. The linearity of the associations was evaluated graphically by linear splines with
168 the boundaries placed at the quartiles (Greenland 1995a). The analysis was carried out
169 separately for cereal products known to contain AR (rye bread, crisp bread, refined bread,
170 beer) and for dietary fiber (total- and cereal fiber). Associations were expressed per 100g/d for
171 cereal food components and per 10 g/d for dietary fiber. Spearman's rank correlation
172 coefficients were calculated for selected variables. The GLM procedure in SAS version 9.1
173 for Unix (SAS institute, NC, USA) was used for statistical analysis.

174

175

176 **RESULTS**

177 Base-line characteristics of women were calculated according to quartiles of plasma total AR
178 (**Table 1**). The plasma AR homologue concentration for combined cases and controls were
179 skewed to the right for all individual homologues and their sum (**Figure 1**). The median total
180 plasma AR concentration was 78 nmol/l and inter quartile range was 106.9 nmol/l. The
181 mean±SD in the same variable was 115±112 nmol/l.

182

183 The average intake of products identified as likely to contain AR was 133±62 g/d (range 0.5-
184 368 g/d) and included only bread. Among these product categories, rye bread was the
185 dominant source, accounting for 51±24 % whereas 13±17% was refined bread, 4.6± 8.9 %
186 crisp bread and 31±25% "other bread". Additionally, daily intake of beer and fat derived from
187 animal carcasses were investigated. The intake of beer was 77±146 g/d and animal fat intake
188 was 15±6 g/d. The FFQ contained one question on intake of oats/muesli, but since this
189 question is likely to reflect oats to a large extent and because oats do not contain AR, this
190 question was omitted to avoid misclassification.

191

192 All plasma AR homologues were correlated to rye bread intake ($r= 0.21-0.31$, $P<0.0001$)
193 whereas only C17:0 and total plasma AR concentration was correlated to crisp bread intake
194 ($r= 0.11$, $P=0.03$ and $r= 0.12$, $P<0.03$, respectively) (**Table 2**). Some AR homologues were
195 significantly correlated to total dietary fiber intake and all were correlated to cereal fiber
196 intake ($r= 0.16-0.20$, $P<0.001$) (Table 2). No significant correlation between non-fasting
197 serum cholesterol and plasma AR concentration was found (Table 2).

198

199 Three different regression models (univariate, multivariate with dietary predictors,
200 multivariate with dietary and non-dietary predictors) were tested in order to find the one that
201 explained most of the variation in plasma AR concentration. In most cases, inclusion of
202 potential predictors other than AR containing foods, such as BMI, serum cholesterol (non-
203 fasting), time since last meal and energy intake did not affect the estimates or the variation
204 explained by the model and hence, these were omitted in the final models. When testing food
205 items likely to contain whole grain in the final model, rye bread was the only significant
206 determinant of plasma total AR concentration ($P<0.0001$) (**Table 3**). An increase of rye bread
207 intake of 100 g/d was associated with a raise of plasma total AR concentration of 87 % (46-
208 139%, 95% CI) ($P<0.0001$). Results were the same for the individual AR homologues with an
209 associated increase of plasma AR homologue concentration of 69-114 % ($P<0.0001$) for
210 homologues C17:0- C25:0 per 100 g/d increment of rye bread intake. The final models,
211 including all identified potential food sources of AR simultaneously, explained 8-12 % of the
212 total variation of plasma concentration of individual AR homologues and 9% of total AR
213 concentration (Table 3).

214

215

216

217 Total dietary fiber was a significant predictor of total plasma AR concentration (P=0.04), but
218 not for individual AR homologues (**Table 4**). Only about 1 % of the total plasma AR
219 concentration was explained by the model. When dietary fiber was divided into cereal fiber
220 and non-cereal fiber, a 10 g/d increase in cereal fiber intake was associated with a 36%
221 increase in plasma total AR concentration (P=0.011) (Table 4). The associated increase was of
222 the same magnitude for the individual AR homologues, and was significant for all
223 homologues except for C25:0. About 2% of the total variation in plasma AR concentration
224 was explained by the models.

225

226

227 **DISCUSSION**

228 This is the first study where determinants of plasma AR concentration were investigated.
229 Samples used were from an endometrial cancer case-cohort study conducted within the
230 Danish “Diet, Health and Cancer” cohort (Olsen et al 2009)..

231

232 Plasma total AR concentration in the present study, where non-fasting samples were used,
233 was considerably higher and showed a wider range than what was reported in a recent Finnish
234 study (n=54) where fasting samples in free-living subjects were analyzed (115 ± 112 nmol/l
235 compared to 65 ± 34 nmol/l) (Aubertin-Leheudre et al 2008).

236

237 Among commonly consumed foods in the Western diet, AR are mainly present in whole grain
238 and bran products of wheat and rye (Chen et al 2004, Landberg et al 2005, Mattila et al 2005,
239 Ross et al 2003). Small amounts are also found in barley and in very small amounts in some
240 refined products due to contamination or a high extraction rate in the milling process
241 (Landberg et al 2008a, Ross and Kochhar 2009, Zarnowski et al 2001). From the FFQ used in

242 the study, rye bread, white bread, crisp bread, and “other bread” were identified as food
243 categories likely to contain AR. Rye was expected to be the main predictor because of the
244 high whole grain content typically found in Danish rye bread (Mejborn et al 2008) and due to
245 about twice as much AR in whole grain rye compared to whole grain wheat (Chen et al 2004,
246 Mattila et al 2005, Ross et al 2003) . The spearman rank correlation coefficient between rye
247 bread intake and plasma AR concentration in the present study ($r=0.25$) was lower compared
248 to what was found when comparing total whole grain intake and plasma AR concentration in
249 a recent study in free-living subjects in Switzerland (Ross et al 2009) and about the same as
250 what was found between rye bread intake and plasma concentration in a Finnish intervention
251 study ($r=0.34$) (Linko et al 2005). The higher correlation coefficient in the Swiss study is
252 likely due to the fact that the FFQ used in that study was designed to capture whole grain
253 intake specifically whereas a general FFQ was used in the present study. Neither crisp bread
254 nor “Other bread” were independent predictors of plasma AR concentration, which is
255 probably due to the large variation in whole grain content in these categories and a small
256 contribution of AR intake from these foods. The observed correlation between crisp bread and
257 plasma C17:0 concentrations is probably due to confounding by rye bread intake because the
258 association disappeared when mutually adjusting for the other food variables. As expected,
259 white bread was not associated with an increase in plasma AR since it contains no, or only
260 very small amounts of (Chen et al 2004, Ross and Kochhar 2009). Additionally, beer and fat
261 from animal sources were tested, because small amounts of AR have been detected in beer
262 samples as well as in adipose tissue from pigs and humans (Jansson et al 2010) (unpublished
263 data), but without showing any association with plasma AR concentration.

264

265

266

267 In an earlier experimental study, it was suggested that plasma AR concentration should be
268 adjusted for serum cholesterol value in order eliminate variation caused by differences in
269 carriers, since AR are transported in lipoproteins (Linko-Parvinen et al 2007). However, in the
270 present study, no correlation between serum cholesterol and AR homologues or the sum of
271 them was observed, giving no support to this adjustment. The lack of a correlation may be due
272 to the fact that serum cholesterol was determined in non-fasting samples or that plasma AR
273 concentration is unaffected by difference in serum-cholesterol concentrations. In the present
274 study, no significant correlation between plasma AR concentration and total energy intake,
275 BMI, or time since last meal was found (Table 2) and hence, in most cases, models adjusting
276 for these variables did not affect associations between intake and AR concentrations (Table 3-
277 4). The finding of no association between plasma AR concentration and energy intake in the
278 present study is probably due to fact that AR are present in foods with low energy density
279 (products mainly classified in the rye bread category).

280

281 It was possible to explain about ≈ 10 % of the variation in plasma AR concentration by the
282 final models with cereal product intake, whereas only about 1-2 % of the variation was
283 explained by the models with dietary fibre. The amount of explained variation was in the
284 same range as found for serum ENL in different studies (Horner et al 2002, Johnsen et al
285 2004, Kilkkinen et al 2001, Milder et al 2007). In the present study, a large proportion of the
286 unexplained variation is probably due to the use of non-fasting samples. The half-life of
287 plasma AR has been estimated to about 5 h (Landberg et al 2006, Landberg et al 2008d), and
288 hence the within-subject plasma concentration over the day will be highly affected by the
289 intake frequency. However, a rather long absorption time will dampen fluctuations around
290 steady state, given a frequent intake. There was no improvement by including time since last
291 meal in the models, probably since time since last meal did not efficiently correct for the time

292 since last AR containing food intake. Another likely source of unexplained variation is the
293 inter-individual differences in absorption and elimination kinetics. Moreover, the FFQ used in
294 the present study was designed with the focus on the ability to rank subjects according to
295 intake of foods potentially important for cancer aetiology (Tjønneland et al 1991). However,
296 only 4 (6 if including beer and animal fat) food-variables were used for the intake estimation
297 of foods including whole grain likely to contain AR. Since both the whole grain content in
298 products and AR concentration in whole grain varies considerably (Chen et al 2004, Mattila et
299 al 2005), the estimate is likely to be subjected to a considerable measurement error.
300 Additionally, subjects may not have been able to distinguish between artificially coloured
301 dark bread and dark bread containing whole grain leading to additional measurement errors
302 with resulting underestimation of the value of plasma AR as biomarkers of whole grain
303 intake.

304

305 Cereal fiber intake was significantly correlated to plasma total AR in the present study
306 (Spearman's $r = 0.19$, $P < 0.001$). This correlation was weaker than observed in a recent survey
307 in 56 free living Finnish subjects (Pearson's $r = 0.41$, $P < 0.0003$) in which fiber intake was
308 assessed by 3-day food record (Aubertin-Leheudre et al 2008) and plasma AR was analysed in
309 a sample taken during the food record period. Since plasma AR have a rather short half-life, a
310 single sample is expected to reflect short- to medium term intake (Landberg et al 2008d), and
311 hence reflects the same time period as the food records. The FFQ, on the other hand, is
312 expected to reflect long-term average intake. The underlying true correlation between plasma
313 AR concentration and intake estimated by FFQ is probably stronger than the observed, since
314 no adjustment for measurement errors in the biomarker was undertaken (Rosner and Willett
315 1988).

316

317 In conclusion, the results of the present study demonstrate that the rye bread intake was the
318 major determinant of plasma AR concentration in the studied population, among foods and
319 non-dietary variables tested. At a nutrient level, both total- and cereal fiber were recognized as
320 significant determinants. However, these variables only explained a small proportion of the
321 total variation in plasma AR concentration, probably due to misclassification of whole grain
322 intake by the FFQ and because of poor precision in the biomarker due to measurement error
323 inherent to short half-life and the use of non-fasting samples. Future studies to evaluate the
324 usefulness of AR as biomarkers in epidemiological studies should focus on determination of
325 long.-term reproducibility and to what extent plasma concentration reflect whole grain intake
326 in fasting and non-samples in different free-living populations.

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329

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333 for the analysis of AR in plasma and drafted the manuscript. JC was main responsible for the
334 statistical analysis. AT and KO were the founders of the cohort. All authors critically
335 evaluated the manuscript before submission and RL was primarily responsible for the final
336 content. None of the authors have any have any advisory board affiliation, financial
337 agreements or other relationships that might have led to a conflict of interest.

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Table 1 Base-line subject characteristics of 360 (n=176 endometrial cancer cases and n=184 subcohort members) from the Danish Diet, Cancer and Health cohort¹

Characteristics	All subjects (n=360)
Age (y)	56 (53-60)
BMI (kg/m^2)	25 (22.7-28.6)
Energy (kJ/d)	8396 (7168-9863)
Rye bread	62.5 (62.5-62.5)
“Other bread”	31.4 (5.7-100.0)
Crisp bread	1.0 (0.2-5.1)
Refined bread	4.3 (0.5-30.0)
S-cholesterol ² (mmol/L)	6.6 (5.3-6.8)

¹ Values reported are medians and interquartile range (25th-75th percentile).

² Non-fasting samples of serum cholesterol

Table 2: Spearman's rank correlation¹ between plasma AR homologue concentration and dietary and non-dietary factors among 360 Danish women, of which 176 were diagnosed with endometrial cancer.

Variable	Plasma AR homologue concentration					
	(nmol/l)					
	C17:0	C19:0	C21:0	C23:0	C25:0	Total
Rye bread (g/d)	0.31***	0.26***	0.21***	0.25***	0.26***	0.25***
White bread (g/d)	-0.07	-0.06	-0.05	-0.06	-0.07	-0.06
Crisp bread (g/d)	0.11*	0.09	0.08	0.08	0.05	0.12*
Other bread (g/d)	0.02	0.11*	0.16**	0.11*	0.06	0.09
Beer (mL/d)	0.06	-0.06	-0.08	-0.11*	0.04	-0.09
Animal fat ² (g/d)	0.05	0.02	0.01	0.01	-0.10	0.02
BMI (kg/m ²)	-0.02	-0.02	-0.01	-0.04	-0.01	-0.01
Time since last meal (h)	0.04	0.04	0.03	0.06	0.06	0.05
Total S- cholesterol(mmol/L)	0.05	0.07	0.05	0.07	0.09	0.07
Total energy intake (KJ/d)	0.06	0.08	0.08	0.08	0.06	0.08
Total dietary fiber (g/d)	0.10*	0.14**	0.14**	0.13**	0.11	0.14**
Cereal fiber (g/d)	0.17**	0.20**	0.20***	0.19***	0.16	0.19***
Other fiber (g/d)	0.00	0.04	0.05	0.05	0.03*	0.05

¹ Significant correlation coefficients are indicated with stars, where * P<0.05, ** P<0.01 and *** P<0.001.

² Fat from animal sources

Table 3 Food determinants of plasma AR homologue C17:0, C19:0, C21:0, C23:0, C25:0 and total AR concentration (nmol/l) for 360 postmenopausal women¹.

	Univariate analysis	Full model ²	Final model ³	P ⁴
<i>Whole grain food items⁵</i>				
<i>Rye bread</i>				
C17:0	106 (62;164)	124 (72;193)	114 (67;173)	<0.0001
C19:0	88 (46;141)	105 (57;168)	96 (52;151)	<0.0001
C21:0	62 (26;109)	74 (33;129)	69 (31;117)	<0.0001
C23:0	85 (43;141)	96 (48;161)	91(47;149)	<0.0001
C25:0	106 (54;175)	117 (59;198)	110 (57;181)	<0.0001
Total AR	81 (41;131)	94 (-0.1;153)	87 (46;139)	<0.0001
<i>Crisp bread</i>				
C17:0	0.07 (-9;8)	2 (-19;11)	-19 (-36;2)	0.91
C19:0	-5 (-13;4)	-3 (-11;6)	-4 (-11;5)	0.39
C21:0	-7 (-14;102)	-5 (-13;4)	-5 (-13;3)	0.20
C23:0	-5 (-13;4)	-3 (-11;7)	-4 (-12;5)	0.38
C25:0	-3 (-12;7)	-0.1 (-10;10)	-2 (-11;8)	0.68
Total AR	-5 (-12;4);	-3 (-11;38)	-3 (-11;5)	0.40
<i>White bread</i>				
C17:0	-21 (-45;14)	-11 (-38;29)	-19 (-43;14)	0.23
C19:0	-16 (-41;21)	-3 (-34;41)	-13 (-39;24)	0.44
C21:0	-11 (-0.4;29)	2 (-30;49)	-6 (-34;34)	0.72
C23:0	-11 (-39;30)	0.1 (-32;49)	-8 (-36;33)	0.66
C25:0	-18 (-46;24)	-10 (-42;40)	-18 (-45;24)	0.36
Total AR	-15 (-40;22)	-4 (-33;40)	-12 (-38;24)	0.47
<i>Other bread⁶</i>				
C17:0	-11 (-28;9)	-1 (-21;23)	-7 (-24;12)	0.43
C19:0	4 (-15;27)	15 (-8;44)	7 (-12;31)	0.48
C21:0	12 (-9;36)	21 (-4;52)	15 (-6;40)	0.17
C23:0	2 (-17;26)	12 (-12;42)	-4 (-12;5)	0.57
C25:0	-6 (-26;19)	4 (-20;35)	-2 (-22;23)	0.84
Total AR	3.6 (-15;26)	13 (-9;41)	7 (-12;30)	0.48
<i>R²</i>				
C17:0	-	0.12	0.12	-
C19:0		0.11	0.10	
C21:0		0.09	0.08	
C23:0		0.09	0.09	
C25:0		0.09	0.08	
Total AR		0.11	0.09	

¹ Estimated change (%) of plasma total AR concentration corresponding to a 100 g/d increase in intake of food items. Within parentheses, the 95% CIs of the estimates are presented.

² Model included all dietary factors and adjustment for BMI, serum cholesterol (non-fasting), total energy intake, time since last meal.

³ Model included all dietary factors but excluded non-dietary factors.

⁴ P-values are shown for variables in the final model

⁵ Whole grain foods likely to contain AR were included

⁶ Bread that could not be classified as rye bread, crisp bread or white bread.

Table 4: Fiber determinants of plasma AR homologues C17:0, C19:0, C21:0, C23:0, C25:0 and total AR concentration (nmol/l) for 360 postmenopausal women¹

	Univariate analysis	Full model ²	Final model ³	P ⁴
<i>Nutrient</i>				
<i>Total dietary fiber</i>				
C17:0	8 (-6;23)	12 (-8;36)	-	0.26
C19:0	13 (-1;29)	19 (-2;45)	-	0.08
C21:0	15 (-0.5;31)	21 (-1;47)	-	0.07
C23:0	15 (-0.2;32)	22 (-0.4;51)	-	0.05
C25:0	13 (-3; 33)	25 (-1;57)	-	0.11
Total AR	14 (0.3;30)	22 (1;49)	-	0.04
<i>Cereal Fiber</i>				
C17:0	36 (7;73)	62 (17;127)	38 (8;76)	0.01
C19:0	41 (11;79)	70 (22;139)	41 (11; 80)	0.01
C21:0	39 (10;77)	64 (18;29)	39 (9;77)	0.01
C23:0	36 (6;75)	61 (14; 128)	35 (5; 74)	0.02
C25:0	31 (-1;73)	63 (10-139)	30 (-2; 72)	0.07
Total AR	37 (9;74)	65 (19;128)	36 (8; 73)	0.01
<i>Non-cereal fiber</i>				
C17:0	-21 (-45;14)	1 (-18;25)	-6 (-22; 12)	0.46
C19:0	3 (-14;23)	8 (-12;34)	-0.6 (-17; 19)	0.94
C21:0	7 (-11;27)	11 (-10;37)	3 (-14; 23)	0.75
C23:0	8 (-10;30)	14 (-9; 42)	4 (-13; 26)	0.65
C25:0	8 (-12;32)	16 (-9;48)	5 (-15; 29)	0.66
Total AR	7 (-10;27)	13 (-8; 39)	3 (-13; 23)	0.72
<i>R²</i>				
C17:0	-	0.031	0.019	-
C19:0		0.039	0.021	
C21:0		0.035	0.021	
C23:0		0.022	0.016	
C25:0		0.028	0.011	
Total AR		0.023	0.019	

¹ Estimated change (%) of plasma total AR concentration corresponding to a 10 g/d

increase in intake of fiber component. Within parentheses, the 95% CIs of the estimates are presented. Data from both cases and controls were included, after testing for any differences.

² Model included cereal- and non-cereal fiber and were adjusted for BMI, serum cholesterol (non-fasting), total energy intake, time since last meal. R^2 are reported for models where cereal fiber and non-cereal fiber were added in the same model.

³ Model included cereal- and non-cereal fiber but excluded non-dietary factors. R^2 are reported for models where cereal fiber and non-cereal fiber were added in the same model.

⁴ P-values are shown for variables in the final model except for total dietary fiber, where P-values are from the univariate analysis.

Figure 1: Frequency distributions of plasma AR homologues C17:0, C19:0, C21:0, C23:0, C25:0 and total AR concentration (nmol/l) for 360 postmenopausal women.

